Secondary lymphoid organ development

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General Introduction

Secondary lymphoid organs such as spleen and lymph nodes are crucial for long-term defense against dangerous pathogens because it is in these tissues of the vertebrate body that immunological memory can be formed [1 - 4]. Immunological memory, the capacity to swiftly and forcefully react to an earlier encountered pathogen, even many years after the first encounter, is the hallmark of our immune system. It is based on the selective differentiation of T and B-lymphocytes into memory lymphocytes during the course of an immune response against a pathogen [5 - 7]. This process is best studied in the case of B-lymphocytes. For these cells it is described that the B cells that produce the most specific antibodies will selectively differentiate in specific sites of the lymphoid organs [8].

As a consequence of its development in the bone marrow, from where they will migrate to the lymphoid organs, each individual B lymphocyte has a unique recognition site on its immunoglobulin antigen receptor as a result of differential gene usage [9, 10]. Upon activation after antigen encounter in the lymphoid organs, a part of the B cells that become memory cells undergo further differentiation, during which random point mutations occur in the genes that encode the recognition domain of the immunoglobulins [11 - 13]. When this leads to the production of an immunoglobulin with enhanced binding properties, the B cell can proliferate and form a clone of B cells producing an immunoglobulin of high specificity for the particular antigen [14 - 18].

In secondary lymphoid organs B cells reside in follicles and here the selection of memory B cell clones will take place [19 - 24]. The differentiation involves active proliferation leading to prominent changes in the histological appearance of the B cell follicles that are called germinal centers [25]. The existence of a germinal center is therefore an indication that B cell selection upon antigen encounter is taking place or has recently taken place [8, 26, 27]. In addition to pathogens resulting from an infection [28], self-antigens can also trigger immune responses and cause B cells to form autoantibodies, a proces which is seen during autoimmune diseases [29]. In part of the patients that suffer from an autoimmune disease, tertiary lymphoid tissues containing autoantibody producing plasma cells or germinal centers are found at sites of chronic inflammation [30 - 34]. Although these lymphocyte aggregates and germinal center-like structures may be a secondary phenomenon due to chronic inflammation [35], it is clear that lymphoid tissue does not always protect the individual from disease, but can also be formed as a consequence of disease.

Previously, different research groups have shown in animal models that a targeted deletion of specific genes partially or completely blocks the development of secondary lymphoid tissues [36 - 56]. Analyses of all these mutant mice increased the awareness that there are permissive events in the development of these tissues that allow their formation at predefined locations in the body. A part of these events concerns the differentiation of hematopoietic progenitors towards mature B and T lymphocytes, which need to populate the secondary lymphoid tissues in order for these organs to function properly. However, in these mutants the anlagen of secondary lymphoid organs can still form [57]. In addition, there are events that are instructive for the development of the stromal environment that harbors these immune cells [58, 59, 60]. Because this environment forms the basis of the lymphoid tissue, the events that govern its development are elementary for lymphoid tissue formation. Expanding

the fundamental knowledge of these processes can thus help to understand how lymphoid tissue is formed, both in normal organ development and in pathology.

To contribute to this knowledge, we have focussed on early events in lymph node and splenic white pulp development. Despite the large record of studies on lymphoid tissue development, most of these studies involve research in either the development of other lymphoid tissues such as the thymus or Peyer's patches, or in rather late events in lymph node or splenic white pulp development. The aim of this study is to identify the cell types involved in the earliest stages of murine lymph node and splenic white pulp development and to further unravel the molecular mechanisms that are required for the formation of these secondary lymphoid organs.

Over the years the research on lymphoid tissue formation has provided scientific knowledge about key players in lymph node development, such as lymphoid tissue inducer (LTi) cells and stromal organizer cells [43, 44, 46, 47, 61 - 66]. These findings and other milestones in the field of lymphoid tissue development are reviewed in chapter 2. But additional characterization of the role of these and other cells in the earliest steps of lymph node development was still necessary to further unravel the elementary processes that are required for lymph node development. In chapter 3 we have investigated what subpopulations of cells constitute the lymph node primordium at developmental time point E16.5 in murine lymphoid organ development. Here we also further defined the expression pattern of stromal organizer cells and we have shown that these can be subdivided in two subsets that are differentially distributed between distinct types of lymph nodes.

It was already shown previously that the triggering of the LTβR on stromal organizer cells by LTi cells is a crucial event in the process of lymph node formation [43, 44]. In chapter 4 we studied which molecules are upregulated upon ligation of the LTβR, apart from cell adhesion molecules (CAMs) and chemokines. In addition, we investigated the presence of different cell types at stage E14.5 in murine lymph node development and we present data that show that the earliest occurrences in this development are independent of LTβR signaling.

To unravel the contribution of lymphatic endothelial cells (LECs) that comprise the lymphatic system and form the capsule of lymph nodes, the influence of Prox-1 deficiency on the lymph node organogenesis was studied (chapter 5). Prox-1 is crucial for the development of LECs and a deficiency in this homeobox gene blocks the development of lymphatics. Because the current dogma that is based upon hundred-year-old findings states that lymph nodes develop out of lymph sacs, structures formed by the lymphatic system early in development, a block in lymphangiogenesis would block lymph node development as well, thus implying that LECs are the true inducers of lymph node formation. However, our data firmly disprove this model, and therefore other inductive signals must initiate lymph node development (chapter 5).

We further extend our analysis of lymph node development in chapter 6. Here we underline the robustness of lymphoid organogenesis, since this process can still take place when the signaling required for normal angiogenesis is severely disturbed. In this chapter the effect of a deficiency in C5-epimerase on lymphoid organogenesis is investigated for the first time. The enzyme C5-epimerase is

required for the normal processing of heparan sulphate proteoglycans (HSPGs). These molecules are present on cell surfaces and in the extracellular matrix where they bind a large variety of molecules, such as cytokines, chemokines and growth factors. Despite the importance for many signaling molecules to bind to normally processed HSPGs for their function, the generation of lymphoid tissue is not abolished in C5-epimerase deficient animals. However, the severe abnormalities seen in especially lymph nodes and spleen of these animals point to an important role of C5-epimerase in lymphoid organ morphogenesis.

Although spleen and lymph nodes are quite distinct organs, the white pulp, the lymphoid compartment of the spleen is often compared to a lymph node because of its structural and functional resemblance. However, when we investigated the development of the spleen in more detail, important differences appear. Because the role of LTi cells in the induction of splenic white pulp was subject to discussion, we sought to identify which cells were responsible for the LTβR signal in the splenic white pulp. In chapter 7 we investigated in depth the role of both LTi and B cells in the induction of splenic white pulp development and have identified a subset of white pulp stromal cells that express the molecules that are required to attract and retain these cells.

The results from these chapters and the implication for future research in view of the recent literature are discussed in chapter 8.

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